REMARKS/ARGUMENTS

Claims 3-5 and 19-22 currently appear in this application. The Office Action of June 19, 2003, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

The Present Invention

The present invention is directed to a method for determining if a human or other animal has been exposed to a hemolysin-producing fungus or, if an environmental sample is contaminated by a fungus. The method depends on the presence of fungal hemolysin(s) in the sample or alternatively, antibodies produced against the hemolysins, by the human or other animal as the basis for the determination.

This method can be used for many purposes, including diagnosing problems or diseases associated with possible fungal exposures. Also, this method could be useful in monitoring contamination of environmental samples or buildings with fungi.

In the first format of the method, the actual

hemolysin(s) can be measured in serum or other bodily fluids or tissues, such as tracheal secretions, bronchiolar lavage fluid, nasal lavage fluid, sinus tissues, secretions, etc or environmental sample. This type of test is particularly useful if the human or animal has only been exposed to the hemolysin-producing fungus for a short period of time or if the exposure is of an immunodeficient or imunocompromised individual, e.g. human infant or AIDS patient, respectively. This method also teaches that contamination by fungi in environmental samples, e.g. dust, food, water etc can be evaluated by measuring hemolysin(s). In all applications, any of number of conventional analytical procedures can be used to make the measurement.

There are many standard procedures or techniques for measuring proteins. The hemolysin(s) *per se* can be measured by GC-MS, MALDI, ELISA etc., or any other conventional assay for proteins. The present application gives an example of an ELISA measurement, but it is recognized that this is solely for purposes of illustration. Any other method of detecting or measuring hemolysin can be used.

In the second format of the method, the antibodies (IgG, IgE, etc.) produced by an exposed human or other animal after exposure to the hemolysin-producing

fungus are measured. The second format of the method actually measures the exposure of an animal to a hemolysin-producing fungus by measuring antibodies made by the exposed human or other animal. Any of number of conventional analytical procedures can be used to make the measurement.

For example, a purified hemolytic protein might be used to capture the specific antibodies present in, *e.g.*, serum or other bodily fluids such as nasal secretions. By measuring the antibodies against hemolysin(s), the types of fungi in the exposed animal's environment can be determined. Antibodies can be measured by using any convenient technique including radioactive or fluorescent tracers, affinity columns etc.

Usefulness of the Information in the Patent Application

The first format of the method, measuring hemolysin(s) directly, is most useful in studying immediate exposures. For example, an employee enters a moldy building and alleges that he has been sickened by exposure to a fungus. If he has indeed been exposed to a fungus, the hemolysin may be measurable in his blood or other bodily fluids or tissues. In a single exposure, there would be no time for antibodies to be produced, but the hemolysin itself is measurable. The hemolysin can be

measured by any standard analytical procedure applicable to proteins. One example of this is an immunoassay.

Similarly, any type of environmental sample, including but not limited to, dust, water, food etc, can be tested for fungal contamination by testing for the presence of the fungal hemolysin(s). The same type of analytical procedures for measuring hemolysin(s), described above, could be used for these types of samples, also. The value of such analyses could include, but are not limited to, preventing exposures to heavily contaminated dust, testing food or bottled water for fungal contamination, etc.

The second format of the method, measuring antibodies to the hemolysin(s), is more useful in measuring long term exposure. For example, an employee works in a moldy building for a long period of time. This type of exposure results in antibody production after weeks or months of exposure. Measuring the antibodies to/against fungal hemolysin(s) would thus make it possible to estimate a human's long term exposure to a fungus which produces the hemolysin. This knowledge might help diagnose conditions like asthma or allergy.

Rejections under 35 U.S.C. 112

Claims 3-5 and 19-21 are rejected under 35 U.S.C. 112, second paragraph, for failing to particularly

point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleges that the claims are indefinite in reciting "antibodies to fungal hemolysin" and "active fragments thereof."

This rejection is respectfully traversed. First of all, hemolysin is defined here as a protein/polypeptide which can cause some degree of lysis of red blood cells. The fungi producing an hemolysin include, but not limited to: *Absidia coerulea/glauca; Absidia corymbifera; Acremonium strictum; Alternaria alternata; Apophysomyces elegans, Saksenea vasiformis; Aureobasidium pullulans; C. albicans, C. tropicalis, C. krusei, C. parapsilosis, C. glabrata* and *C. lusitaniae; Chaetomium globosum; Cladosporium cladosporioides-* Type 1; *Cladosporium cladosporioides-* Type 2; *Cladosporium herbarum; Cladosporium sphaerospermum; Conidiobolus coronatus/incongruus; Cunninghamella elegans; Eurotium amstelodami/chevalieri/herbariorum/rubrum/repens; Epicoccum nigrum; Memnoniella echinata; Mortierella polycephala/wolfii; Mucor mucedo; Mucor amphibiorum/circinelloides/heimalis/indicus/mucedo/racemosus/ ramosissimus; Rhizopus azygosporus/homothalicus/microsporus/oligosporus/oryzae; Myrothecium verrucaria; Rhizomucor meihei/pusillus/variabilis; Rhizopus stolonifer;*

Scopulariopsis asperula; Scopulariopsis brevicaulis/fusca; Scopulariopsis brumptii; Scopulariopsis chartarum; Scopulariopsis sphaerospora; Stachybotrys chartarum; Trichoderma asperellum/hamatum; Trichoderma asperellum/hamatum/viride; Trichoderma harzianum; Trichoderma longibrachiatum/citroviride; Trichoderma viride*/atroviride/koningii; Ulocladium atrum; Ulocladium chartarum; Ulocladium botrytis; Wallemia sebi; Aspergillus auricomus; Aspergillus awamori ; Aspergillus caesiellus; Aspergillus caespitosus; Aspergillus candidus, Aspergillus carbonarius,; Aspergillus cervinus; Aspergillus clavatus; Aspergillus flavipes; Aspergillus flavus; Aspergillus fumigatus; Aspergillus giganteus; Aspergillus niger; Aspergillus niveus; Aspergillus ochraceus; Aspergillus ostianus; Aspergillus oryzae; Aspergillus paradoxus; Aspergillus parasiticus; Aspergillus sojae; Aspergillus penicillioides; Aspergillus puniceus; Aspergillus restrictus; Aspergillus sclerotiorum; Aspergillus sulfurous; Aspergillus sydowii; Aspergillus tamarii; Aspergillus terreus; Aspergillus unguis; Aspergillus ustus; Aspergillus versicolor; Aspergillus wentii; Emericella nidulans (Aspergillus nidulans); Emericella quadrilineata; Emericella rugulosa; Emericella variecolor; Eurotium amstelodami (Aspergillus*

amstelodami); *Eurotium chevalieri*; *Eurotium herbariorum*; *Eurotium repens*; *Eurotium rubrum*; *Neosartorya fischeri*; *Paecilomyces varioti*; *Penicillium aethiopicum*; *Penicillium atramentosum*; Penicillium aurantiogriseum; *Penicillium brevicompactum*; *Penicillium canescens*; *Penicillium camembertii*; *Penicillium chrysogenum* svar. I; *Penicillium chrysogenum* svar. II; *Penicillium citreonigrum*; *Penicillium citrinum*; *Penicillium commune*; *Penicillium coprophilum*; *Penicillium corylophilum*; *Penicillium crustosum*; *Penicillium decumbens*; *Penicillium digitatum*; *Penicillium echinulatum*; *Penicillium expansum*; *Penicillium fellutanum*; *Penicillium glabrum*; *Penicillium glandicola*; *Penicillium griseofulvum*; *Penicillium hirsutum*; *Penicillium implicatum*; *Penicillium islandicum*; *Penicillium italicum*; *Penicillium janthinellum* svar. I; *Penicillium janthinellum* svar. II; *Penicillium lividum*; *Penicillium melinii*; *Penicillium miczynskii*; *Penicillium olsonii*; *Penicillium oxalicum*; *Penicillium polonicum*; *Penicillium purpurogenum*; *Penicillium raistrickii*; *Penicillium restrictum* svar. I; *Penicillium restrictum* svar. II; *Penicillium roquefortii*; *Penicillium sclerotiorum*; *Penicillium simplicissimum*; *Penicillium solitum*; *Penicillium spinulosum*; *Penicillium thomii*; *Penicillium tricolor*;

Penicillium variabile; Penicillium verrucosum svar. I; *Penicillium verrucosum* svar. II; *Penicillium viridicatum; Penicillium waksmanii.*

When a human or other animal has been exposed to a fungus which produces a hemolysin, hemolysin is incorporated in the bodily fluids, *e.g.*, by breathing in the fungal spores or mycelia, by growth of the fungus in the sinuses, or other such means. After a period of exposure to fungal hemolysin (which is an antigen to this human or other animal), the human or other animal's body produces antibodies to/against the fungal hemolysin.

It is because the animal's body produces antibodies to/against the fungal hemolysin that these antibodies can be measured, resulting in a method for determining whether the human or other animal has been exposed to the fungus. This can be done, for example, by capturing the antibodies using the fungal hemolysin itself (or fragment thereof). This could be done in any number of standard methods including, but not limited to, affinity specific capture columns, ELISA, etc.

"Active Fragments of Fungal Hemolysin"

With respect to the definition of "active fragments of fungal hemolysin", this has been defined in the specification as filed, beginning at paragraph 0040 on page 10. It is clear from this description of active

fragments that one skilled in the art can readily determine, without undue experimentation, if a protein or fragment thereof, is an active fragment, *i.e.*, if it has the same or equivalent antigenic properties as the entire fungal hemolysin. Thus, the "fragment of the hemolysin" can be used as an equivalent to the use of the entire hemolysin in the applications described in this application.

It should be clear from the specification that the antibodies bind to the fungal hemolysin (or fragment thereof), since the fungal hemolysin is an antigen. Claim 3 has been amended to recite that the fungal hemolysin(s) are detected; the fungal hemolysins in this case are antigenic or immunogenic in the animal, but the term "antigen" has been cancelled from the claims.

Art Rejections

Claims 2-3 and 19-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sakaguchi et al. in view of Harlow et al.

This rejection is respectfully traversed. First, the abstract of Sakaguchi et al merely describes that asp-hemolysin was measured in animal tissues after the spores of the fungus, *Aspergillus fumigatus,* were injected into mice. Also, they demonstrate that asp-

hemolysin can cause lesions and is involved in the pathogenicity of this fungus.

This team of researchers in Japan has been studying asp-hemolysin and publishing their results since 1962. They have published more than 25 papers, abstracts etc. Never have they suggested the use of any fungal hemolysin, including asp-hemolysin, as a technique for measuring fungal exposures and occurrence as described in the present application. Their interest and publications have strictly been in the realm of understanding the *A. fumigatus* infection process including more recently, the binding of the asp-hemolysin and the possible role in cholesterol metabolism. If there had been any suggestion of the applications taught in this patent i.e. if it were "obvious", these authors surely would have published them in the past 40 years.

The Harlow et al. reference merely describes antibody binding that is the basis for immunoassays. The present application is not claiming any particular method of measuring the hemolysin(s) or anti-bodies to the hemolysin(s) (or fragment thereof). The method of detection of either the hemolysin (which is an antigen) or the antibodies to hemolysin(s) is immaterial. Any conventional detection method can be used, although immunoassay is described in the specification as one

example. Thus Harlow et al. is irrelevant to this patent application.

As the Federal Circuit stated in *In re Lee*, 61 USPQ2d 1430 (January 18, 2002, Fed. Cir.), "As applied to the determination of patentability *vel non*, when the issue is obviousness, 'it is fundamental that rejections under 35 U.S.C. 103 must be based on evidence comprehended by the language of that section.' *In re Grasselli*, 53 USPQ2d 1769, 1774 (Fed. Cir. 2000)… When patentability turns on the question of obviousness, the search for an analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness *See, e.g., McGinley v. Franklin Sports, Inc*, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001) ('the central question is whether there is a reason to combine [the] references,' a question of fact drawing on the *Graham* factors."

'The factual inquiry whether to combine references must be thorough and searching.' *Id.* This precedent has been reinforced in myriad decisions, and cannot be dispensed with, *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris, Inc.,* 56 USPQ2d 1456, 1459 (Fed. Cir. 2000). ('a showing of a suggestion, teaching, or motivation to combine the prior art

references is an "essential component of an obviousness holding"') (quoting *C. R. Bard, Inc. v. M3 Systems, Inc.* 48 USPQ2d (Fed. Cir. 1998)) The Court went on to quote *In re Dembiczak*, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999), "Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."

There is a requirement for specificity in combining references, *See, In re Kotzab*, 55 USPQ2d 13134, 1317 (Fed. Cir. 2002)("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.").

In the present case, the Examiner has shown no motivation to combine the cited references to arrive at the particular invention claimed herein. Sakaguchi et al. (or any of the other articles, abstracts etc published by this group of researchers in Japan) provide no suggestion of using such a detection method to determine if an animal has been exposed to or that an environmental sample has been contaminated with a fungus. Harlow et al. merely describe immunochemical techniques, with no suggestion that these techniques can be used to

detect fungal hemolysins or why detecting fungal hemolysins is important. As noted above, the method of measurement is not critical to the present invention. The present invention provides a method for determining if an animal has been exposed to a hemolysin-producing fungus by detecting the hemolysin or antibodies produced in response thereto. This invention is neither taught nor disclosed in any of the cited art.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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